526 Rec'd PCT/PTO 19 SEP 2000

FORM PTO-1390 (REV 11-98)	U.S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	ATTORNEY'S DOCKET NUMBER 39-219
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		U S APPLICATION NO (If known, see 37 C F R 1.5) 09/646532 (To Be Assigned)
INTERNATIONAL APPLICATION NO. **PCT/GB99/00727**	INTERNATIONAL FILING DATE **March 19, 1999**	PRIORITY DATE CLAIMED **20 March 1998**

TITLE OF INVENTION
ADP-GLUCOSE TRANSPORTER OF THE AMYLOPLAST

APPLICANT(S) FOR DO/EO/US
EMES et al

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. A copy of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☒ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)).
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☒ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has **NOT** expired.
   d. ☐ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. To 16. Below concern document(s) or information included:

11. ☒ An Information Disclosure Statement under 37 C.F.R. 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 C.F.R. 3.28 and 3.31 is included.
13. ☒ A FIRST preliminary amendment.
    ☐ A SECOND or SUBSEQUENT preliminary amendment.
14. ☐ A substitute specification.
15. ☐ A change of power of attorney and/or address letter.
16. ☒ Other items or information. **PTO-1449/ International Search Report/ Three References**

459390

532 Rec'd PCT/PTC 19 SEP 2000

U.S. APPLICATION NO. (If known, see 37 C.F.R 1.5) (To Be Assigned) 09/646532	INTERNATIONAL APPLICATION NO PCT/GB99/00727	ATTORNEY'S DOCKET NUMBER 39-219

17. ☒ The following fees are submitted:

BASIC NATIONAL FEE (37 C.F.R. 1.492(a)(1)-(5):

-- Neither international preliminary examination fee (37 C.F.R. 1.482) nor international search fee (37 C.F.R. 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO $970.00

-- International preliminary examination fee (37 C.F.R. 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO $840.00

-- International preliminary examination fee (37 C.F.R. 1.482) not paid to USPTO but international search fee (37 C.F.R. 1.445(a)(2) paid to USPTO $690.00

-- International preliminary examination fee paid to USPTO (37 C.F.R. 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) $670.00

-- International preliminary examination fee paid to USPTO (37 C.F.R. 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) $96.00

						CALCULATIONS		PTO USE ONLY
ENTER APPROPRIATE BASIC FEE AMOUNT =						$	840.00	
Surcharge of $130.00 for furnishing the oath or declaration later than ☐ 20 ☒ 30 months from the earliest claimed priority date (37 C.F.R. 1.492(e)).						$	130.00	
CLAIMS	NUMBER FILED		NUMBER EXTRA	RATE				
Total Claims	16	-20 =	0	X	$18.00	$	0.00	
Independent Claims	3	-3 =	0	X	$78.00		0.00	
MULTIPLE DEPENDENT CLAIMS(S) (if applicable)				$260.00		$	0.00	
TOTAL OF ABOVE CALCULATIONS =						$	**970.00**	
Reduction by ½ for filing by small entity, if applicable. A Small Entity Statement must also be filed (Note 37 C.F.R. 1.9, 1.27, 1.28).							0.00	
SUBTOTAL =						$	**970.00**	
Processing fee of $130.00, for furnishing the English Translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 C.F.R. 1.492(f)). +							0.00	
TOTAL NATIONAL FEE =						$	**970.00**	
Fee for recording the enclosed assignment (37 C.F.R. 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 C.F.R. 3.28, 3.31). **$40.00** per property +						$	0.00	
Fee for Petition to Revive Unintentionally Abandoned Application ($1210.00 – Small Entity = $605.00)						$	0.00	
TOTAL FEES ENCLOSED =						$	**970.00**	
						Amount to be: refunded	$	
						Charged	$	

a. ☒ A check in the amount of $970.00 to cover the above fees is enclosed.
b. ☐ Please charge my Deposit Account No. 14-1140 in the amount of $_____ to cover the above fees. A duplicate copy of this form is enclosed.
c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 14-1140. A duplicate copy of this form is enclosed.
d. ☐ The entire content of the foreign application(s), referred to in this application is/are hereby incorporated by reference in this application.

NOTE: Where an appropriate time limit under 37 C.F.R. 1.494 or 1.495 has not been met, a petition to revive (37 C.F.R. 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

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Mary J. Wilson
NAME

32,955 **September 19, 2000**
REGISTRATION NUMBER Date

459390

09/646532
532 Rec'd PCT/PTC 19 SEP 2000

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of

EMES et al Atty. Ref.: **39-219**

Serial No. **(To Be Assigned)** Group:

National Phase of **PCT/GB99/00727**

Filed: **September 19, 2000** Examiner:

For: **ADP-GLUCOSE TRANSPORTER OF THE AMYLOPLAST**

September 19, 2000

Assistant Commissioner for Patents
Washington, DC 20231
Sir:

PRELIMINARY AMENDMENT

Prior to calculation of the filing fee and in order to place the above identified application in better condition for examination, please amend the claims as follows:

IN THE CLAIMS

Claim 3, line 1, delete "or 2",
Claim 4, line 1, change "any one of claims 1-3" to --claim 1--,
Claim 12, line 1, delete "or 11".

REMARKS

The above amendments are made to place the claims in a more traditional format.

Respectfully submitted,

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101 Rec'd PCT/PTO 19 SEP 2000

09/646532

ADP–GLUCOSE TRANSPORTER OF THE AMYLOPLAST

The present invention relates to an ADPglucose transporter protein which regulates starch biosynthesis in plants and more particularly to the modulation of the transporter protein for the purposes of, for example, producing modified starches and/or increasing starch yield. The invention also relates to methods of modulating such biosynthesis and also to genetically modified plants and plant cell lines in which starch biosynthesis is modulated.

Starch biosynthesis in plants is the major determinant of yield in agricultural production of cereals. On a world basis cereals contribute 50% of total dietary energy supplies, and up to 75% of the human daily calorific intake is starch. Starch also has a broad range of industrial applications (e.g. in manufacture of paper, paint and adhesives). Variation in the chemical structure of starch, determined by the ratio of amylose to amylopectin and the degree of branching in amylopectin in the starch polymer, can significantly alter the properties of starch and the regulation of such structure is highly desirable for optimising the industrial application in which starch is being used.

In plant storage tissues the biosynthesis of starch occurs in specialised cellular organelles called amyloplasts. The immediate soluble substrate for starch synthesis is the sugar nucleotide ADPglucose (ADPG). Amyloplasts must import the carbon required for starch synthesis and we have previously demonstrated that amyloplasts of wheat endosperm are capable of synthesising starch from either exogenous sugar (hexose) phosphates and ATP, or by direct uptake of ADPG. Recent evidence has demonstrated that in cereal endosperm the vast proportion of the enzyme which synthesises ADPG is located in the cytoplasm (outside the amyloplast).

We have performed studies with amyloplasts which show that ADPG supports rates of starch synthesis 30-fold greater than from hexose-phosphate uptake.

Furthermore we have found during the active period of grain filling nearly all of the enzyme activity responsible for the synthesis of ADPG resides in the cytoplasm. This lead us to realise that the major source of carbon taken up by the amyloplast for starch synthesis is ADPG.

As synthesis of ADPG is located in the cytoplasm, the transport protein responsible for uptake of ADPG into amyloplasts must therefore play a pivotal role in regulating starch synthesis. The ADPG transporter influences not only starch yield, but also quality (a commercially important aspect of this raw material) since the starch synthases involved in amylose and amylopectin synthesis have different affinities for ADPG.

The present invention is founded on our identification of a protein which is integral to the membrane of amyloplasts (e.g. amyloplasts from developing wheat endosperm) and which, we have established, is the ADPG transporter. The protein is in the inner membrane of amyloplasts and may be obtained by the procedure of Example 1 (see below).

The ADPG transporter protein may be characterised by the following features:

(a) an amino acid sequence selected from:

SMPLNAAVKM	(SEQ ID NO. 1)
GAXXXETAWACGXA	(SEQ ID NO. 2)
NFRYTNFAX	(SEQ ID NO. 3)
GATXGNXAHAMG	(SEQ ID NO. 4)
SVLWTEXXDXXXGFR	(SEQ ID NO. 5)
VXLAPXNP	(SEQ ID NO. 6)
PYNXAYQDXG	(SEQ ID NO. 7)

(wherein X indicates any amino acid and the other letters represent the conventional single letter code for amino acids); and

(b) a molecular weight of about 38 kDa.

A protein comprising any of the amino acid sequences listed under (a) above represents a new protein and according to a first aspect of the present invention there is provided an ADPglucose transporter protein, or a modification or fragment thereof capable of ADPglucose transport activity comprising at least one amino acid sequence selected from the group of:

(i)	SMPLNAAVKM;	(SEQ ID NO. 1)
(ii)	GAXXXETAWACGXA;	(SEQ ID NO. 2)
(iii)	NFRYTNFAX;	(SEQ ID NO. 3)
(iv)	GATXGNXAHAMG;	(SEQ ID NO. 4)
(v)	SVLWTEXXDXXXGFR;	(SEQ ID NO. 5)
(vi)	VXLAPXNP; and	(SEQ ID NO. 6)
(vii)	PYNXAYQDXG.	(SEQ ID NO. 7)

The protein of the first aspect of the invention preferably comprises two or more of the amino acid sequences (i) – (vii) and may contain all such sequences. When the protein contains any of the sequences (v), (vi) or (vii) they may be present in multiple copies.

It is preferred that the protein contains at least one of the amino acid sequences (i) – (iii). The protein may contain two, or all of the amino acid sequences (i) – (iii).

The protein preferably has a molecular weight in the region of 35kDa – 43kDa and more preferably a molecular weight of approximately 38 kDa.

The protein may be isolated from membranes of amyloplasts from cereal endosperm (e.g. spring wheat endosperm).

By "modifying" or "controlling" the amount and / or activity of a protein in accordance with the first aspect of the invention in a starch producing plant (as compared to the amount which is present in a wild-type plant) it is possible to modulate starch production in various desirable ways. Thus according to a second aspect of the present invention there is provided a method of regulating starch production in plants or plant cells comprising modulating the activity of the protein according to the first aspect of the invention.

According to a first embodiment of this method the activity of the ADPG transporter may be decreased in amyloplast membranes of plant cells. A decrease in transporter activity will favour production of starch with a higher proportion of amylopectin resulting in the production of "waxy" starches which can be exploited as thickening agents in food and coatings. By "waxy" we mean a starch with a lower proportion of amylose relative to amylopectin.

A decrease in activity can be achieved by exogenous addition of an agent which suppresses transporter activity (i.e. an ADPG transporter antagonist or a neutralising species raised against the transporter). Alternatively an agent may be used which increases the rate of breakdown of active transporter or one which decreases expression of the protein (e.g. protein synthesis inhibitors, inhibitors of post-translational modification. ribozymes, antisense RNA or DNA, inhibitory transcription factors etc).

According to a second embodiment of this method the activity of the ADPG transporter is increased in amyloplast membranes of plant cells. This favours increased starch yield and will favour production of starch with a higher proportion of amylose. Increasing amylose content increases the viscosity and gel strength of starch pastes.

Such starches can be exploited in the production of biodegradable plastics. High amylose content also influences gelatinization and retrogredation properties (crystallisation of cooling starch components following heating) of starches. Since retrogredation of amylopectin is a factor influencing the rate at which baked foods go stale, we have found that baked foods comprising starches with an increased proportion of amylose go stale less quickly and therefore have longer shelf lives. For instance, bread comprising starches containing an increased proportion of amylose go stale less quickly than bread containing unmodifed starch.

An increase in activity can be achieved by exogenous addition of an agent that increases activity of the transporter according to the first aspect of the invention (i.e. an ADPG transporter agonist or an activating species). Alternatively an agent may be used which decreases the rate of breakdown of active transporter.

The activity may also be promoted by increasing the amount of transporter in the amyloplast membrane. This may be achieved by application of the protein to a plant or plant cell in a form in which the protein will be taken up and incorporated into the membranes of the starch producing organelles of the plant. However it is preferred that the amount of transporter in the amyloplast membrane is increased by promoting expression of the protein. This may be achieved by increasing the activity of transcription factors which promote gene expression (e.g. by administering to a plant cell the transcription factors *per se* or by administering phytohormones or other agents which promote the activity of these transcription factors).

It is most preferred to increase ADPG transporter expression by genetically manipulating a plant cell.

Starch production is preferably regulated according to the first or second embodiments of the method of the invention by genetically manipulating plant material. Such manipulation requires the use of a DNA molecule that codes for protein capable of

modulating ADPG transporter activity. Thus according to a third aspect of the invention there is provided a DNA molecule encoding for a protein which directly or indirectly modulates ADPG transporter protein activity, said DNA molecule being capable of being transcribed to lead to the expression of said protein.

Said protein directly or indirectly has activity for modulating ADPG transporter protein activity such that starch production from a plant cell is regulated. By "directly" we mean that the protein coded by the DNA molecule *per se* has the required activity for regulating starch production. By "indirectly" we mean that the protein coded by the DNA molecule undergoes or mediates (e.g. as an enzyme) at least one further reaction to provide an agent effective for regulating starch production.

A preferred DNA molecule according to the third aspect of the invention codes for a protein of the first aspect of the invention. Using such DNA molecules, it is possible to produce genetically modified plant material having an enhanced capability for the production of the protein of the first aspect of the invention so as to modulate starch production according to the second embodiment of the second aspect of the invention. These preferred DNA molecules are particularly useful for transforming a plant cell such that it will contain multiple copies of genes encoding proteins of the first aspect of the invention. Expression of the protein from such multiple copies may give rise to increased copies of proteins with transporter activity greater than is found in unmanipulated plant cells.

It will be appreciated that the base sequence of DNA molecules according to the third aspect of the invention may exhibit some base variability but still code for the same, or a functionally equivalent protein. For instance, DNA molecules which encode for the ADPG transporter or a modification or fragment of such a protein which retains ADPG transport activity, may have base changes which would not alter the amino acid sequence of the transporter expressed from the DNA molecule (e.g. due to redundancy

in the genetic code). Variants of the DNA molecule may even encode a protein with altered amino acid sequence which nevertheless retains transporter's function.

It is preferred that DNA molecules according to the third aspect of the invention are propagated within a suitable DNA vector to form a recombinant vector. The vector may for example be a plasmid, cosmid or virally based (e.g Gemini virus vectors). Such recombinant vectors are of utility when replicating the DNA molecule. Furthermore recombinant vectors or derivatives thereof are highly useful for transforming plant cells or protoplasts.

The recombinant vectors will frequently include one or more selectable markers to enable selection of cells transformed with the DNA vector and, preferably, to enable selection of cells harboring the recombinant vectors that incorporate the DNA molecule of the third aspect of the invention. Examples of such selectable markers include genes conferring resistance to kanamycin, G418, phosphinothricin, ampicillin or neomycin.

Recombinant vectors may also include other functional elements. For instance, the recombinant vector may be designed such that the vector and DNA molecule integrates into the genome of a cell. In this case DNA sequences which favour targeted integration (e.g. by homologous recombination) are desirable. Alternatively recombinant vectors can be designed such that the vector will autonomously replicate in the cytosol of the cell. In this case, elements which induce DNA replication may be required in the recombinant vector. Preferred recombinant vectors which autonomously replicate in the cytosol of the cell will be capable of existing in a host cell in multicopies. Multiple copies of such vectors are useful for increasing expression of protein encoded by the DNA molecule of the third aspect of the invention above levels obtainable in normal wild-type plant cells (i.e. cells which have not been genetically modified).

The recombinant vector may also further comprise a promoter or regulator to control expression of the gene as required. Furthermore the vector may also comprise a transcription terminator such as the nopaline synthase (nos) terminator).

The promoter may be an inducible promoter such as AlcR/ALcA (as disclosed in WO 93/21334) or the GST system. A typical inducer for the AlcR/ALcA promoter which may be used is cyclohexanone. Inducers such as cyclohexanone may be sprayed onto crops which have been genetically modified with DNA coding for the AlcR/ALcA promoter operatively linked to a DNA molecule which directly or indirectly modulates ADPG transport in order that starch production may be regulated according to the methods of the invention. Commercially available safeners may be used in conjunction with inducers such as cyclohexanone (typically at doses in the range of 1Kg/ha).

A plant may be grown in the absence of an activator of the inducible promoter and at a predetermined time (e.g. when seed endosperm is developing in cereals or tubers developing in potatoes) the foliar parts of the plant may be exposed to the activator of the promoter (e.g. by crop spraying) to regulate starch production. Activators may be applied to the transformed plants typically 6 - 96 hours, preferably 12 - 72 hours and most preferably 12 - 48 hours before starch production needs to be regulated.

Starch production may be closely regulated by spraying plants with the activator of the promoter at suitable times. It will be appreciated that optimal spraying times will depend upon the type of starch required (highly branched or minimal branching) and the species of plant used. By way of example only, spraying of cereal crops may be any time following the onset of flowering (approximately1 day post-anthesis) up to maturity of the grain (approximately 60-70 days post-anthesis).

It will be appreciated that constitutive promoters may also be used according to the invention (e.g. the CaMV35s promoter from the cauliflower mosaic virus).

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Furthermore tissue specific promoters such as the Gliadin promoter (normally found in seeds) or the Patatin promoter (found in potato) may be used.

Other promoters which may be linked to DNA coding for proteins which directly or indirectly regulate ADPG transport are well known in the art. For instance, the promoters reviewed by Gatz (Annu Rev Plant Physiol Plant Mol Biol 48 p89-108, 1997) may be used to transform a cell according to the second aspect of the invention.

Preferred recombinant vectors may be formed using plasmids such as pBI 101, Bin 6, Bin 19 or pUC or derivatives of such vectors.

The recombinant vectors may be transferred to plant cells or protoplasts by, transformation, transfection, infection, microinjection, cell fusion, protoplast fusion or ballistic bombardment. For example, transfer may be by ballistic transformation with coated gold particles, agrobacterium mediated transformation, liposomes containing the DNA molecule, viral vectors (e.g. Gemini virus) by electroporation, by chemical transformation (e.g. treating cells with calcium chloride and Polyethylene glycol favours DNA uptake) or by means of direct DNA uptake (e.g. endocytosis).

The DNA molecule according to the third aspect of the invention may be delivered, by the abovementioned methods, to a plant cell or protoplast to be transformed without it being incorporated in a vector.

Plant material genetically modified with a DNA molecule according to the third aspect of the invention represents a fourth aspect of the invention, according to which there is provided a plant cell transformed with the DNA molecule of the third aspect of the invention. Such transformed cells are particularly useful for regulating starch production according to the method of the second aspect of the invention.

Many different types of plant material may be treated according to the method of the second aspect of the invention or transformed according to the fourth aspect of the invention. The material may be a growing plant, plant tissue, a plant cell line, a seed or any other suitable source of plant material which is capable of growth. If desired plant tissue may be removed from the treated plant material for further cultivation. This tissue may be in the form of an embryo, a growing shoot tip, a bud or a root tip from a plant or growing leaf cuttings. Such tissue may be cultivated in soil or may be grown *in vitro*. *In vitro* grown tissue may be grown under suitable conditions to give rise to a plant or alternatively the tissue may be grown to develop a plant cell line.

Plants to which the invention is applicable for modulating starch production include, but are not limited to, cereals (e.g. wheat, barley, rye, oats, maize, rice and sorghum), tubers (e.g. potatoes), root crops (e.g. sugar beet, carrots, turnips, swedes and cassava), legumes (e.g. peas, beans) and forage foodstuffs for animals (e.g. grasses).

Cell lines which have been transformed according to the fourth aspect of the invention may be developed from such plants.

The plant material, plants and plant cell lines should be maintained or propagated in a suitable growth medium. When plants are grown in the ground, the environment may represent a suitable medium, although supplementation with phytohormones, vitamins and/or carbohydrate etc may be required. Plant material, plants and plant cell lines propagated *in vitro* require a suitable growth medium to sustain the cells.

Such plant material (plants, plant tissue or plant cell lines) may, for example, be produced by transforming plant cells (e.g. the embryo) and growing plants from said transformed cells. The plants may be grown and seeds collected following selfing of said plants. Alternatively progeny of the transformed cells may be collected by *in vitro*

propagation of said transformed cells. The transformed cells may be propagated using essentially standard culturing techniques which are known in the art of plant science.

The invention is illustrated by the following non-limiting Example.

EXAMPLE 1

Protocol for Purification of the ADPG Transporter from Amyloplasts

Plant Material and Amyloplast Isolation

Spring wheat (*Triticum aestivum* L. Cv. Axona) was grown under the conditions described by Tetlow *et al.* (1993). Endosperm tissue was obtained from developing grains taken from the mid-ear region of the head 8-14 days after anthesis (the first appearance of anthers). The dissected endosperm (25-37g fresh weight) was used to prepare amyloplasts mechanically using the method described by Tetlow *et al.* (1994).

Isolation of Organelle Membranes

Purified amyloplasts were ruptured in a buffer containing 100mM tricine-NaOH (pH 7.8), 1mM EDTA, 1mM dithiothreitol, 1mM phenylmethylsulphonyl fluoride (PMSF) and 100μM each of leupeptin, bestatin, pepstatin, 3,4-dichloroisocoumarin, chymostatin, 1,10-phenanthroline, phosphoramidon, and pefabloc (rupturing buffer). All subsequent procedures were performed at 2-4°C. The ruptured amyloplasts were centrifuged for 10 min at 10,000g to remove starch and debris. The supernatant was then centrifuged for 20 min at 100,000g to pellet the envelope membranes. The membrane pellet was resuspended in rupturing buffer containing 1.2M NaCl and left on ice for 30-60 min to remove extrinsic membrane proteins. The NaCl-washed membranes were pelleted (20 min, 100,000g) and the supernatant discarded, before being resuspended in rupturing buffer. The washed membranes were then finally pelleted (20 min, 100,000g) and rinsed three times in rupturing buffer before solubilization (below).

Membrane Solubilization and Protein Fractionation

The washed amyloplast envelope membranes were solublized by resuspension in column running buffer (10mM Tricine-NaOH (pH 7.6), 0.2% (w/v) n-dodecylmaltoside, 1mM PMSF), followed by the addition of 40% (w/v) n-dodecylmaltoside, to give a final

detergent concentration of 8%. After incubation on ice for 10 min the solublized membranes were diluted with running buffer to give a final n-dodecylmaltoside concentration of 1.6%. The sample was centrifuged for 5 min at 13,000g to remove insoluble material and the supernatant loaded onto a $1cm^3$ HiTrap (Pharmacia) Q™ column which had been pre-equilibrated in running buffer. After loading the sample, the column was washed in 5 column volumes of running buffer before running a stepped gradient of 0-2M NaCl (in running buffer). The $2x1cm^3$ fraction (containing ADPG transporter activity) eluting at 90mM NaCl was retained and diluted to $10cm^3$ in running buffer. The sample was then loaded on to a $1cm^3$ HiTrap (Pharmacia) Blue™ at a flow rate of $0.5cm^3$ per minute (column pre-equilibrated in running buffer). After loading the sample the column was washed in 5 column volumes of running buffer. The purified transporter was eluted from the column with $3x1cm^3$ of 30mM ATP in running buffer. The purified protein was concentrated down to about $0.5cm^3$ using Amicon™ microcentrifuge filters (10kDa molecular weight cut off).

Reconstitution of the ADPG Transporter

The solubilized ADPG transporter was reconstituted into liposomes using the procedure described by Tetlow *et al.* (1996) to form proteoliposomes. The transporter was orientated such that it pumped ADPG out of the liposome (i.e the reverse orientation to that found in the amyloplast where ADPG is taken into the amyloplast). Transport of ATP, ADPG, AMP and ADP was then determined (also using the procedure described by Tetlow *et al.* (1996)).

Transport of various radiolabelled substrates (1mM) into proteoliposomes (containing the purified protein) was measured when the proteoliposomes were preloaded with various counter-exchange substrates (10mM). Table 1 shows that the transport rate was highest when ADP or AMP were used as substrates and ADPG was used as the counter-exchange substrate. This illustrates that the isolated protein has ADPG transport activity and furthermore acts as an antiporter transporting ADPG in exchange for ADP and AMP.

TABLE 1

SUBSTRATE (1mM)	COUNTER-EXCHANGE SUBSTRATE (10mM)	TRANSPORT RATE (nmol/min/mg protein)
ATP	Buffer	33
ADPG	Buffer	32
ADP	ADPG	99
AMP	ADPG	109

References

Tetlow *et al.* (1993) Planta **189**: p597-600

Tetlow *et al.* (1994) Planta **194**: p454-460

Tetlow *et al.* (1996) Biochem. J. **319**: p717-723.

ART 34 AMDT

CLAIMS

1. An ADP-Glucose transporter protein, or a modification or fragment thereof capable of ADPG transport activity comprising at least one amino acid sequence selected from the group of:

(i)	SMPLNAAVKM	(SEQ ID NO. 1)
(ii)	GAXXXETAWACGXA	(SEQ ID NO. 2)
(iii)	NFRYTNFAX	(SEQ ID NO. 3)

wherein X indicates any amino acid and the other letters represent conventional single letter codes for amino acids.

2. The ADP-Glucose transporter protein according to claim 1 wherein the protein comprises each of the amino acid sequences (i) - (iii).

3. The ADP-Glucose transporter protein according to claim 1 or 2 wherein the protein has a molecular weight of about 38kDa.

4. A DNA molecule encoding for a protein according to any one of claims 1-3, said DNA molecule being capable of being transcribed to lead to the expression of said protein.

5. A plant cell transformed with the DNA molecule according to claim 4.

6. A method of regulating starch production from a plant, plant tissue or plant cell comprising modulating activity of the amyloplast membrane ADP-Glucose transporter protein in said plant, plant tissue or plant cell.

7. The method according to claim 6 wherein the activity of the ADP-Glucose transporter is decreased.

ART 34 AMDT

8. The method according to claim 7 wherein starch is produced with a higher proportion of amylopectin than found in starch from an unmodulated plant, plant tissue or plant cell.

9. The method according to claim 7 wherein "waxy" starches are produced for use as thickening agents in food and coatings.

10. The method according to claim 6 wherein activity of the ADP-Glucose transporter is increased.

11. The method according to claim 10 wherein the yield of starch is increased relative to an unmodulated plant. plant tissue or plant cell.

12. The method according to claim 10 or 11 wherein starch is produced with a higher proportion of amylose than found in starch from an unmodulated plant, plant tissue or plant cell.

13. The method according to claim 12 wherein starch is produced with increased viscosity and gel strength relative to starch from an unmodulated plant, plant tissue or plant cell.

14. The method according to claim 13 wherein the starch is for incorporation in a baked food which goes stale less quickly than baked food containing starch derived from an unmodulated plant, plant tissue or plant cell.

15. The method according to any one of claims 10 - 14 wherein the plant, plant tissue or plant cell is transformed with the DNA molecule according to claims 4.

16. A 38kDa ADP-Glucose transporter protein, capable of ADPG transport activity comprising at least one amino acid sequence selected from the group of:

(i)	SMPLNAAVKM	(SEQ ID NO. 1)
(ii)	GAXXXETAWACGXA	(SEQ ID NO. 2)
(iii)	NFRYTNFAX	(SEQ ID NO. 3)

wherein X indicates any amino acid and the other letters represent conventional single letter codes for amino acids; and

the protein being obtainable by isolating amyloplast membranes, solubilizing said membranes and isolating protein fractions according to the protocol of Example 1.

SEQUENCE LISTING

<110> The Victoria University of Manchester

<120> TRANSPORTER PROTEIN

<130> D088125PWO

<140>
<141>

<160> 7

<170> PatentIn Ver. 2.0

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<400> 7
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Docket No.: 107355

DECLARATION AND POWER OF ATTORNEY UNDER 35 USC §371(c)(4) FOR PCT APPLICATION FOR UNITED STATES PATENT

As a below named inventor, I hereby declare that:
my residence, post office address and citizenship are as stated below under my name;

I verily believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought, namely the invention entitled: PROTEIN EXPRESSION IN FLORAL CELLS

described and claimed in international application number PCT/CA99/00237 filed March 19, 1999 .

I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations §1.56.

Under Title 35, U.S. Code §119, the priority benefits of the following foreign application(s) filed by me or my legal representatives or assigns within one year prior to my international application are hereby claimed:

The following application(s) for patent or inventor's certificate on this invention were filed in countries foreign to the United States of America either (a) more than one year prior to my international application, or (b) before the filing date of the above-named foreign priority application(s):

I hereby appoint the following as my attorneys of record with full power of substitution and revocation to prosecute this application and to transact all business in the Patent Office:

8

James A. Oliff, Reg. No. 27,075; William P. Berridge, Reg. No. 30,024; Kirk M. Hudson, Reg. No. 27,562; Thomas J. Pardini, Reg. No. 30,411; Edward P. Walker, Reg. No. 31,450; Robert A. Miller, Reg. No. 32,771; Mario A. Costantino, Reg. No. 33,565; and Stephen J. Roe, Reg. No. 34,463.

ALL CORRESPONDENCE IN CONNECTION WITH THIS APPLICATION SHOULD BE SENT TO OLIFF & BERRIDGE, PLC, P.O. BOX 19928, ALEXANDRIA, VIRGINIA 22320, TELEPHONE (703) 836-6400.

I hereby declare that I have reviewed and understand the contents of this Declaration, and that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

1 ***Typewritten Full Name of Sole or First Inventor*** 1-CO

	Given Name	Middle Initial	Family Name
	Laurian	S/	ROBERT

2 **Inventor's Signature** Lau— Robert

	Month	Day	Year
3 **Date of Signature**	12	15	00

	City	State or Province	Country
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Note to Inventor: Please sign name on line 2 exactly as it appears in line 1 and insert the actual date of signing on line 3.

IF THERE IS MORE THAN ONE INVENTOR USE PAGE 2 AND PLACE AN "X" HERE ☒

(Discard this page in a sole inventor application)

2-00

Typewritten Full Name of Joint Inventor — Stephen (Given Name) | (Middle Initial) | GLEDDIE (Family Name)

2 **Inventor's Signature:** Stephen Gleddie

3 **Date of Signature:** 12 (Month) | 15 (Day) | 2000 (Year)

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1 *Typewritten Full Name of Joint Inventor* ______________________
Given Name | Middle Initial | Family Name

2 **Inventor's Signature:** ______________________

3 **Date of Signature:** ______________________
Month | Day | Year

Residence: ______________________
City | State or Province | Country

Citizenship: ______________________

Post Office Address:
(Insert complete mailing address, including country) ______________________

1 *Typewritten Full Name of Joint Inventor* ______________________
Given Name | Middle Initial | Family Name

2 **Inventor's Signature:** ______________________

3 **Date of Signature:** ______________________
Month | Day | Year

Residence: ______________________
City | State or Province | Country

Citizenship: ______________________

Post Office Address:
(Insert complete mailing address, including country) ______________________

1 *Typewritten Full Name of Joint Inventor* ______________________
Given Name | Middle Initial | Family Name

2 **Inventor's Signature:** ______________________

3 **Date of Signature:** ______________________
Month | Day | Year

Residence: ______________________
City | State or Province | Country

Citizenship: ______________________

Post Office Address:
(Insert complete mailing address, including country) ______________________

Note to Inventor: Please sign name on line 2 exactly as it appears in line 1 and insert the actual date of signing on line 3.

This form may be executed only when attached to the first page of the Declaration and Power of Attorney of the application to which it pertains.